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Haematological variables of *Oreochromis mossambicus* against *Aeromonas hydrophila* infection by using dissimilar types of gaumutra distillate

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In Aquafarming, gaumutra has been used to recuperate broad healthiness of a specific person. An analysis was conceded out to assess the consequence of different breeds of cow and bull urine (distillate) was used for the analysis of haematological parameters of *Oreochromis mossambicus* fish against *Aeromonas hydrophila* bacterial infection. Average weight (15.6 ± 0.2 g) of healthy fishes was maintained in 70 litres aquaria with most favourable temperature range from 25.8 ± 28.8 ^{o}C. Furthermore, fishes were exposed to different cow urine distillate (CUD) namely, C (without cow urine), T1 (Gir calf), T2 (Gir cow), T3 (Gir Bull calf) and T4 (Gir Bull) on 0.1 % concentration in the medium for seven days. *O. mossambicus* injected with heat-killed *A. hydrophila* (1×10^{-1} cells) during post stimulation phase. Meanwhile, blood samples were collected to determine the different haematological parameters *viz*. total RBCs, WBCs, haemoglobin concentration and mean corpuscular haemoglobin. One-way analysis of variance exhibited significant value ($P<0.01$) of haematological parameters and as well as haematological contents. Based on the above findings, the present study revealed that T2 (Gir cow) urine distillate has the potential antimicrobial activity of *A. hydrophila* and also enhanced the health status of *O. mossambicus*.

[**Keywords:** *Aeromonas hydrophila*; Cow urine; haematology; *Oreochromis mossambicus*; T2- Gir cow]

Introduction

Aquafarming is a unique fast upward nourishment's creating sectors of the universe and intended to enlarge productivity per unit space. Fish farming is considered as an outstanding innovation in the use of organic waste. The manure (cow urine) and supplementary feeding play an imperative role in various types of fish farming practices. The feed and fertilizers cost could be reduced significantly by integrating fish farming with livestock farming which ultimately is economical with respect to the cost of production[1]. The augmented escalation of aquaculture has managed a great number of pathogens, the major cause of the outbreak of diseases in fishes. Therefore, distant predictable methods such as the use of disinfectants and antibacterial medications have been inadequate achievement in the avoidance or therapy of fish infections. The enormous use of antimicrobials for disease resistor and development promotion in fish proliferations the selective pressure used on the microbial world and boosts the usual emergence of bacterial resistance[2]. The use of inoculations in fish farming to avert the bacterial diseases is successful[3], but the main safeguard is species (pathogens) specific and expensive[4].

Recently, cow urine has been reported as a bio enhancer source of allopathic antibiotics and anticancer drugs[1]. It enhanced their effect and reduced the toxic substances and others including adverse effects of their synthetic drugs. As per Ayurveda, cow urine is needed to purify and detoxify in many drugs. Cow urine distillate (CUD) known as "Kamadhenu ark", exhibited many biological activities including immune modulatory and anti-potential anti-microbial effect of various living beings[5]. Freshwater fish *Oreochromis mossambicus* (Tilapia) is often used as a good experimental model and is extensively used in biological, genetic and physiological studies in relation to pollution, stress, or growth promoters[6,7]. Tilapia is an excellent experimental model for haematological studies and microbial infection because of its worldwide economic importance and also tolerates poor water quality conditions. Therefore, the current work was intended to examine the haematological changes and antimicrobial effect of cow urine distillate of T2 (Gir cow) in *Oreochromis mossambicus*.

Materials and Methods

Acclimatization

Oreochromis mossambicus weighing (15.6 ± 0.2 g) and body length (7.8 ± 0.2 cm) of both male and female fishes were bought from the local fish farm, Kumbakonam (Lat. 10.9617° N, Long. 79.3881° E), Thanjavur district, Tamil Nadu. Fishes were carried to the wet lab and adapted for 1 week prior to experimentation. Plastic tubs were washed and then sundried to avoid fungal contamination. Healthy fishes were transferred to plastic tubs (30 liters) containing dechlorinated tap water. They were regularly fed with formulated food and the medium was changed daily to remove faeces and food bits and pieces.

Collection of cow urine

Six disease free cow of each breeds were designated for urine collection. The initial sunrise (4 to 5 am) first urine of Gir calf and Gir bull calf (2 years old) Gir cow and Gir bull (6 years old) was collected from three different sampling stations namely, Goshala, Sri Vittal Rukmini Samsthan, Govindhapuram near Kumbakonam. All cow's urine was collected separately and carried to the lab in sealed antiseptic containers following the methodology of Sattanathan et al[8].

Preparation of cow urine distillate

Dissimilar sex breed Gir goumutra (cow urine) was distillated at 50-60 °C and exhausted by glass multiple distillation apparatus[9].

Experimental setup

After two weeks of acclimatization four groups of fish were treated, each with Gir cow-calf (T_1), Gir cow (T_2), Gir bull calf (T_3) and Gir bull (T4) cow urine distillate at 0.1 % concentration, respectively. A control group was maintained separately without cow urine treatment for seven days.

Preparation of heat-killed whole cell vaccine

A solo colony of *A. hydrophila* from the agar plate was inoculated in the tryptic soy broth. After 24 hrs, the bacterial cells in the broth were exposed to 60 °C for 1 hour in a water bath. The sterility was tested by inoculating a sample on nutrient agar plates. The heat-killed bacterial culture was centrifuged at 3000 rpm for 15 minutes. The supernatant was discarded and the pellet was re-suspended in sterile Phosphate Buffer Saline (PBS). Then the bacterial number was calculated by measuring optical density (OD) in a spectrophotometer and also ascertained by plate count method. The final bacterial concentration was adjusted to 1 x 10^7 cells/ml by serial dilution[10]. The experimental fishes were tested intra peritoneally with the bacterial suspension of 0.2 ml.

Serial bleeding

The Pisces were bled consecutively using 1 ml tuberculin syringe (Glass van) with the 26-gauge syringe from the common cardinal vein situated just below the gills, at systematic intermissions of 7 days after immunization[11]. The blood drawn was collected in heparinized haematological tubes. Great attention was engaged to evade foaming when drawing the plasma into micropipette as this readily resulted in haemolysis[12].

Collection of blood

About 4 -5 ml of blood sample was collected from the fish to use single heparinized one-use syringes containing 0.5 mg ethylene diamine tetra acetic acid (EDTA) as an anticoagulant; correctly mixed and stored at -20 °C for haematological analysis. The blood was stored in -4 °C in the deep freezer prior to analysis.

Blood cell count

The red blood corpuscles (RBC) and white blood corpuscles (WBC) were calculated using haemocyto-meter crystalline chamber using "Hayem's" and "Turch's" diluting fluid, respectively.

Statistical analysis

One-way analysis of variance was executed using Minitab (Version 11: SPPS Inc. Chicago, Illinios USA) software for investigating the significance between mean (MS Excel Microsoft office 2007) was used for graphical presentation of data.

Results and discussion

The changes of haematological parameters like, RBC, WBC, hemoglobin (Hb) and mean corpuscular hemoglobin (MCH) in the fish *Oreochromis mossambicus* both in control were analyzed and also the surveillance rate exposed after the 28th day of the post-immunization process was studied.

Haemoglobin content

Haemoglobin content varied between 5.2 to 7.7 g/dL. The minimum haemoglobin content (5.2 g/dL) was observed in control and the maximum

(7.7 g/dL) content was observed in T3 (Gir bull) (Fig. 1). The response reached its maximum on 14th-day post-immunization as the peak day. Similar findings were reported by Sakthivel[13]. Decreased hematocrit and haemoglobin absorption designate that RBC are being demolished by the leucocytosis action in an erythrocytic anaemia with subsequent erythroblastosis[14]. An increase in hematocrit has been stated as an outcome of oxygen deficiency[15].

Total erythrocyte counts

The total erythrocyte counts of blood sample of the T3 group was found to be the higher and fluctuated significantly ($p < 0.001$) with additional treatment sets. The maximum value of 14.2 $x10^6$ cells/cu mm^{-3} erythrocytes count was observed in T3 and minimum of 11.20$x10^6$ cells/cu mm^{-3} erythrocyte count was observed in control (Fig. 2). Ascribed the reduction in the RBC to heamolytic crisis that effects in cautious anemia in Pisces exposed to heavy metals and herbicides respectively[16,17]. Moreover, the decrease of RBC also primes to growth of hypoxic state which in turn leads to growth in damage of RBC or less in rate of formation of RBC due to non-obtainability of Hb content in cellular medium[18]. The peak day was found to be on the 21st day of post-immunization.

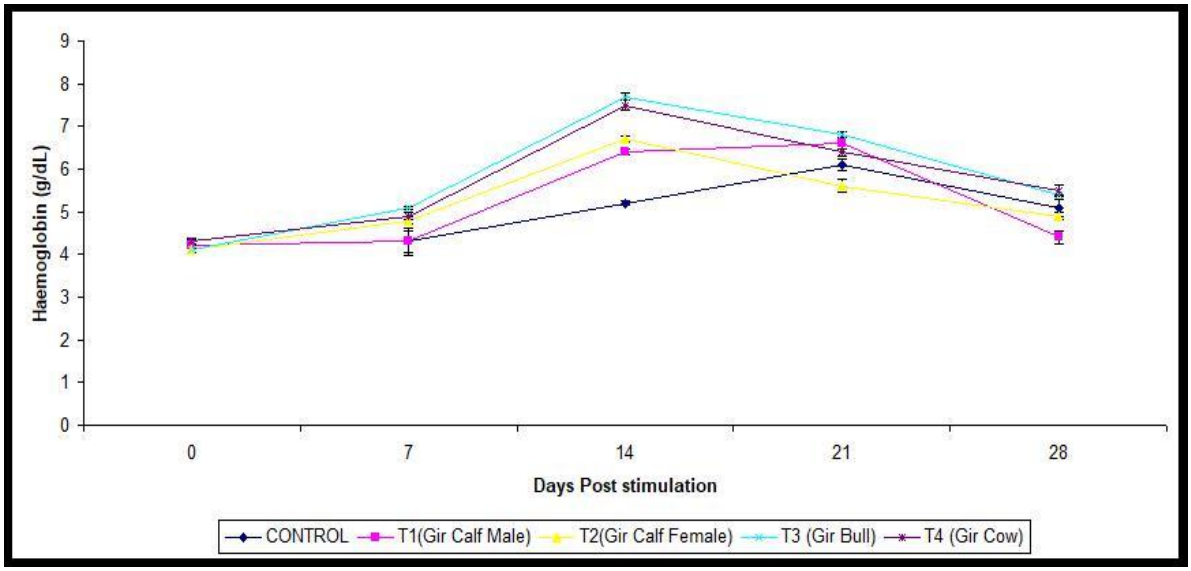

Fig. 1 — Haemoglobin content in *Oreochromis mossambicus* on exposed to dissimilar varieties of gaumutra distillate

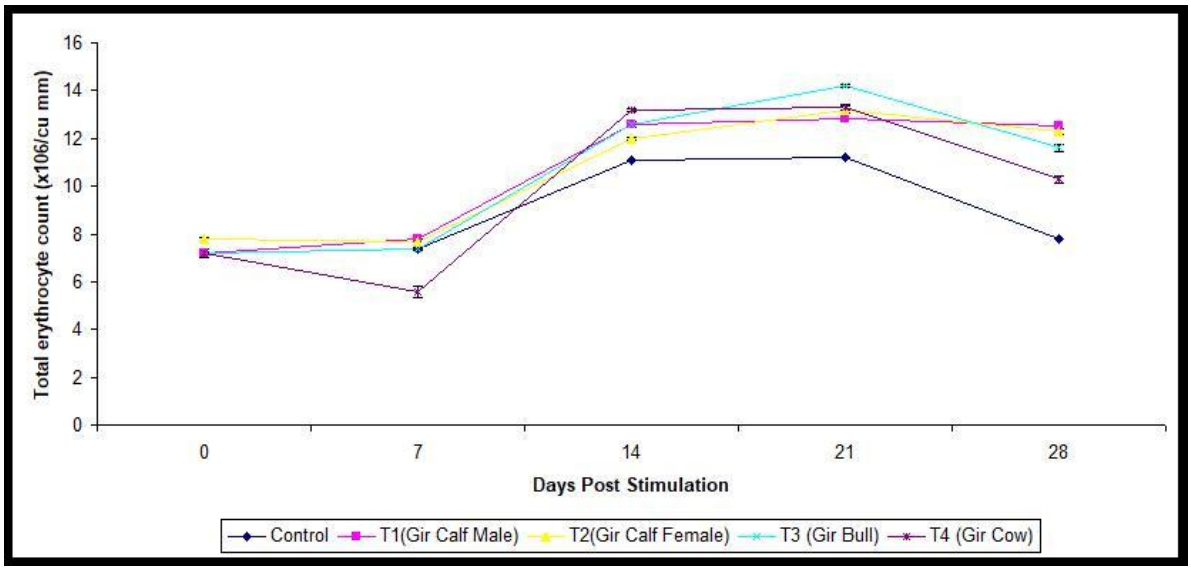

Fig. 2 — Total erythrocyte counts in *Oreochromis mossambicus* on exposed to dissimilar varieties of gaumutra distillate

Mean corpuscular hemoglobin (MCH)

MCH rate of investigational sets was created to diverge expressively ($p>0.05$) when related to control sample (Fig. 3). The blood indices such as MCH, MCV and Mean corpuscular haemoglobin concentration (MCHC) are mainly vital for the finding of anemia in utmost animals[19]. The maximum value was observed in T4 (8.41 %) and the minimum value was observed in T1 (5.96 %).

Total leukocyte counts

Total leuckocyte cells were originated to be the higher value in T2 when compared expressively ($p<0.001$) by control groups T1, T3, and T4. The highest leukocyte counts of $29.6x10^3$/cells cu mm^{-3} were noticed in T2 groups and lowest value of $10.9 x10^3$/cells cu mm^{-3} was noted in control (Fig. 4). Reaction of the defence mechanism of the fish by leucocytosis under pathological conditions and against foreign bodies[20]. A highest activity was found on the 21st daytime of post-immunization.

Relative percentage survival rate

In the current investigation, goumutra of different breeds condensed the death of fishes tested with an infectious strain of *A. hydrophila*. Comparative percentage of existence rate is decreased by altogether treatments (Fig. 5). Control was observed with 66 %, T3 and T4 with 50 % and 75 % protection, respectively while T2 conferred 33 % protection. The measurement of haematological parameters, which are used in this investigation, has provided valuable

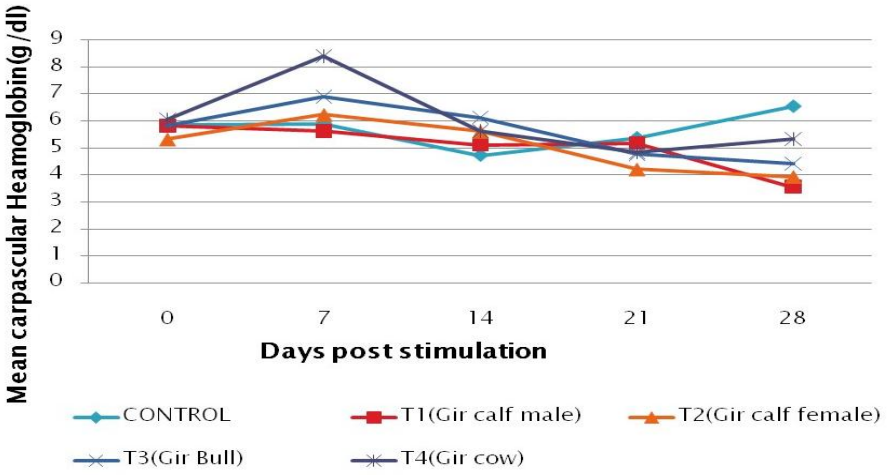

Fig. 3 — Mean corpuscular Haemoglobin content in *Oreochromis mossambicus* on exposed to dissimilar varieties of gaumutra distillate

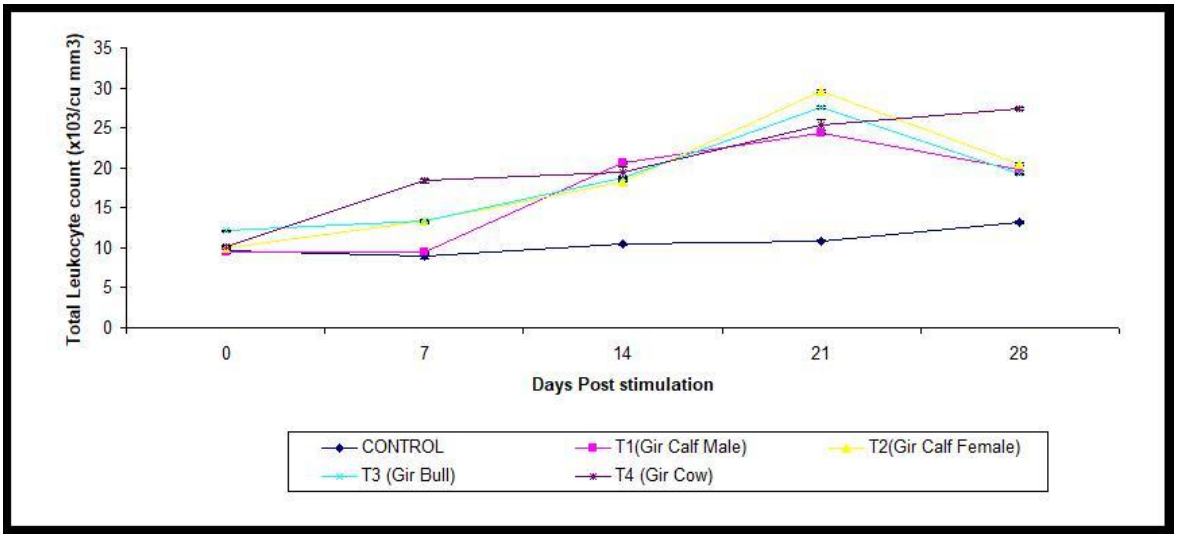

Fig. 4 — Total leukocyte counts in *Oreochromis mossambicus* on exposed to dissimilar varieties of gaumutra distillate

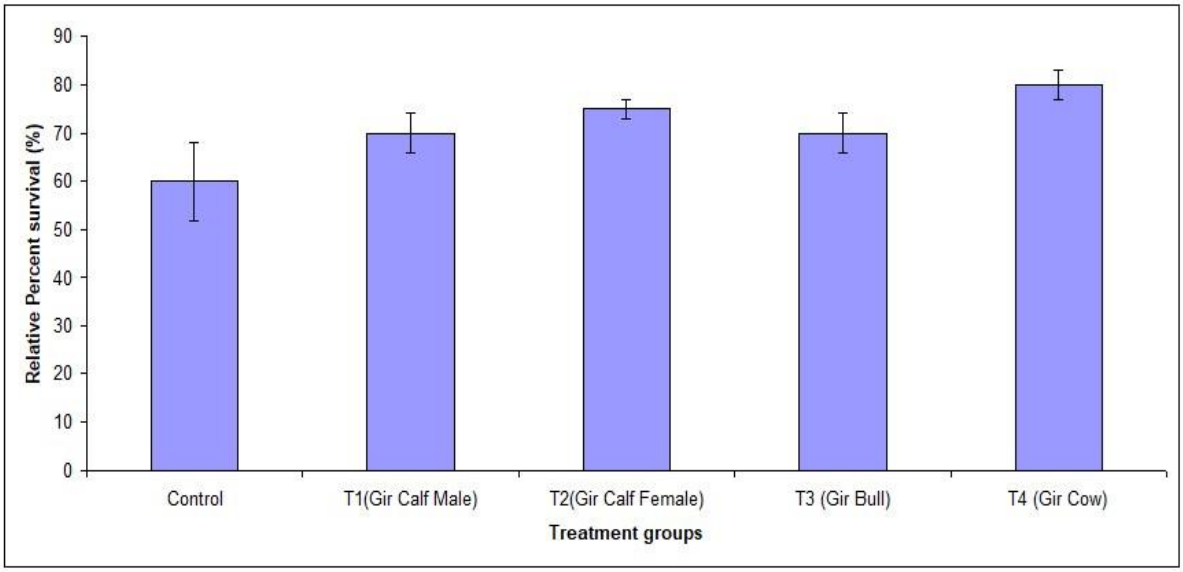

Fig. 5 — Relative percentage survival rate (%) in *Oreochromis mossambicus* on exposed to dissimilar varieties of gaumutra distillate

information which can contribute to the applied and basic research needs of an aquatic field in the assessment of fish health status and in monitoring stress responses like immunization with heat hilled *A. hydrophila*.

At present, tiny is well-known about the haematology of the tilapia *O. mossambicus*, which is unique of the most vital organism in freshwater aquaculture ecosystem; the outcomes accessible above have exposed a stimulating strategy of response on the haematological difference in strained fish. When we compared *O. mossambicus* normal blood standards with those acquired earlier for this species, we found less values of haematocrit (20 %) and RBC count (1.31 $x10^6$ cells/ µL)[21] but obtained somewhat greater values for haemoglobin concentration (6 g/dL). Hrubec et al.[22] Reported even higher haematocrit (33%), RBC count (2.31$x10^6$ cells/ µL) and haemoglobin concentration (8.2 g/dL). In almost all infected fishes, the homeostatic procedures are prolonged beyond the regular limits due to stress[23]. These changes may be due to various strains used in earlier studies and diverse environment and culture settings.

In the fish inserted with the heterotrophic gram-negative bacteria that causes more death in tilapia culture structures, the investigational inoculation only triggered a subclinical contagion and no clinical marks of an infective process were noticed. In agreement through the observation of the corpuscular persistent and the perceive results in the erythrocytes and leukocytes count of blood smears. Similar findings were reported by[24] described microcytic hypo chromic anemia in *Paralichthys olivaceus* due to the reduction of mean corpuscular counts, hypoglobulia and the structural irregularity of the erythrocytes. Subsequently, numerous animals have shown to improve the growth parameters, length, weight, specific growth rate and survival rate in *O. Mossambicus*[25].

Our findings suggest that cultured *O. mossambicus* in cow urine distillate (CUD) against *A. hydrophila* showed significantly ($p<0.001$) improved growth performance when compared with control.

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